PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-355030

(43)Date of publication of application : 10.12.2002

(51)Int.Cl. C12N 1/20
A01N 63/02
//(C12N 1/20
C12R 1:07)

(21)Application number : 2002-094765

(22)Date of filing : 29.03.2002

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(30)Priority

Priority number : 2001099683 Priority date : 30.03.2001 Priority country : JP

(54) METHOD FOR PRODUCING SPORANGIUM OF BACILLUS POPILLIAE, CONTROLLING AGENT AND CONTROLLING METHOD

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a method for efficiently producing the sporangium including the spore of Bacillus popillia and the parasporal body thereof, having activities for controlling insects of Scarabaeidae, and further to provide a controlling agent of the insects of the Scarabaeidae, obtained by the production method, and a controlling method.

SOLUTION: This method is the one for producing the sporangium including the spore and the parasporal body, having the activities for controlling the insects of the Scarabaeidae by culturing the bacteria belonging to the Bacillus popillia. The method comprises culturing the bacteria in a medium containing 0.2-4.0 wt.% glutamic acid and 0.05-0.5 wt.% absorbent. The

controlling agent contains the sporangium including the spore of the Bacillus popillia and the parasporal body obtained by the production method as an active ingredient. The controlling method of the insects of the Scarabaeidae comprises spraying the controlling agent on the soil in which the insects of the Scarabaeidae inhabit.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the manufacture approach of the sporangium containing the spore and the Pallas PORARU body of bacillus POPIRIE which has the prevention effectiveness to the Scarabaeidae insect by cultivating the bacillus belonging to bacillus POPIRIE by the culture medium, the prevention agent of the Scarabaeidae insect, and the prevention approach of the Scarabaeidae insect.
[0002]
[Description of the Prior Art] The larva of the Scarabaeidae insect carries out a diet of the root of wide range vegetation, such as grass, plantation art crops, and a tree, and doing great damage is known. Since the larva of these Scarabaeidae insect lives in the earth, by the chemistry agricultural chemicals sprinkled from the ground, it cannot acquire the prevention effectiveness easily and cannot pinpoint the habitation location of a larva further easily, either. For this reason, in order to make agricultural chemicals permeate a large area in the earth by a lot of crop dusting moreover, we are anxious about the bad influence to natural environment or the body, and it is anxious for the more effective prevention approach.
[0003] The bacillus belonging to bacillus POPIRIE is parasitic on the larva of the Scarabaeidae insect, and makes the symptoms of a milky disease shown, making these die finally is known, and the attempt which is going to use the sporangium of this bacillus for prevention of the Scarabaeidae insect whose chemistry agricultural chemicals cannot be easily effective has been performed for many years. However, although this bacillus was grown in the body of a scarab beetle larva, it was difficult to grow by the culture using an artificial culture medium, and especially the thing for which the sporangium of this bacillus is manufactured by the culture medium was difficult. Moreover, Fukuhara has reported that infection of a larva and pathopoiesis do not take place by the sporangium obtained by the culture which used the culture medium (the page 57 of insect pathology written by Toshihiko Fukuhara, 1979).
[0004] For example, Hines and others has reported the example from which culture of bacillus POPIRIE is tried by the liquid medium containing 1.5% [of yeast extracts], 0.3% [of potassium phosphate], and glucose 0.1%, and 1% of activated carbon, and 2.06x107 sporangia per 1ml of culture medium are obtained at the maximum peptone 0.5% (Journal of Invertebrate pathology, 22 volumes, 377 - 381 pages, 1973). However, the researcher itself has indicated that amino acid composition is not related to production of a sporangium unknown [the rate of glutamic acid to the content or all the amino acid of glutamic acid to a culture medium] (379 pages, the 1st column, the 19th line).
[0005] Moreover, Hines and others has reported having obtained 3.1x107 sporangia per 1ml of culture medium by cultivating bacillus POPIRIE of the liquid-medium component which contains 1% of activated carbon for the cell into which the logarithmic growth anaphase matured peptone (trypton) 0.5%, 1.5% [of yeast extracts], 0.3% [of potassium phosphate], and glucose 0.1% (Journal of Invertebrate pathology, 19 volumes, 125 - 130 pages, 1972). However, this culture approach had long culture time amount, and it had taken about two weeks.
[0006] Moreover, to U.S. Pat. No. 4824671, it cultivates by 1% soluble starch, 0.1% trehalose, 0.5% yeast extract, 0.3% potassium phosphate, and the liquid medium that contains a calcium carbonate 0.1%, and the example from which the number of sporangia of 1x109 per 1ml of culture medium was obtained is given. However, the rate of milky disease infection at the time of the Pallas PORARU body not existing, although the spore was in the sporangium obtained also in this case, but sprinkling a sporangium at a rate of 2.0x1012 pieces in 1kg of soil, and carrying out an ingestion to the larva of the Scarabaeidae insect was 47.59% in seven weeks, and even if compared with the sporangium formed in the larva body, the insect-killing effectiveness over the larva of the Scarabaeidae insect was weak.
[0007]
[Problem(s) to be Solved by the Invention] The technical problem which this invention tends to solve is

to offer the prevention agent and the prevention approach of the Scarabaeidae insect which are acquired by the manufacture approach of obtaining efficiently the sporangium containing the spore and the Pallas PORARU body of bacillus POPIRIE which has the prevention effectiveness to the Scarabaeidae insect, and this manufacture approach.
[0008]
[Means for Solving the Problem] this invention persons showed clearly that the sporangium containing not only the spore of bacillus POPIRIE but a spore and the Pallas PORARU body is required for effective prevention of the Scarabaeidae insect, as a result of repeating research wholeheartedly that the above-mentioned technical problem should be solved. And for production by culture of the sporangium containing this spore and the Pallas PORARU body, it came to complete a header and this invention for it being necessary to cultivate the adsorbent considered to remove glutamic acid and the growth inhibition matter by the culture medium which carried out specific concentration addition.
[0009] 0.05-0.5 mass % That is, this invention is the approach of manufacturing the sporangium which cultivates the bacillus belonging to bacillus POPIRIE by the culture medium, and contains a spore and the Pallas PORARU body, and offers [glutamic acid] the manufacture approach of the sporangium containing the spore and the Pallas PORARU body of bacillus POPIRIE which has the prevention effectiveness to the Scarabaeidae insect characterized by cultivating by the included culture medium for 0.2 to 4.0 mass %, and an adsorbent.
[0010] Moreover, this invention offers the prevention approach of the Scarabaeidae insect which sprinkles the prevention agent and this prevention agent of the Scarabaeidae insect which contain the sporangium containing the spore and the Pallas PORARU body of bacillus POPIRIE obtained by said manufacture approach as an active principle in the habitation soil of the Scarabaeidae insect.
[0011]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. According to the bar JIEIZU manual OBU DETAMI native bacteriology (Bergey's Manual of Determinative Bacteriology) in the bacteriological property of the bacillus belonging to bacillus POPIRIE (Bacillus popilliae) used by this invention, the die length of a gestalt-property is [1.3-5.2 micrometers and width of face] the gram-negative Bacilli which are 0.5-0.8 micrometers, and growth temperature has a spore and the Pallas PORARU body in a sporangium at 20-35 degrees C.
[0012] The sporangium of the bacillus belonging to bacillus POPIRIE is a sac containing the corpuscle called a spore and the Pallas PORARU body (or parasporal body), as shown in the mimetic diagram shown in drawing 1 . However, it was indefinite in whether the word "spore" is a sporangium containing meaning the sporangium which includes only for a spore whether only a spore is meant or the spore, and the Pallas PORARU body in reference by the reference about the culture approach of bacillus POPIRIE using the conventional culture medium. [there are many examples with clear sporangium and spore used fair, and] this invention persons showed clearly that a spore and the Pallas PORARU body are required in order to give the prevention effectiveness by the growth inhibition of insect killing of an insect, especially the Scarabaeidae insect, or a larva.
[0013] in recent years, reclassification of bacillus POPIRIE should be carried out to PAENI bacillus POPIRIE (Paenibacillus popilliae) also including old strain -- ** -- the judgment on the theory of Paterson and others is also shown (Int. J. Syst.Bacteriol., 49 volumes, 1999, 531 - 540 pages), and the treatment of a name is not clear at a present stage. Therefore, in this invention, the bacillus belonging to PAENI bacillus POPIRIE shall also be included with the bacillus belonging to bacillus POPIRIE.
[0014] The culture medium used for the manufacture approach of this invention contains the adsorbent aiming at removal of the matter which checks growth. As this adsorbent, activated carbon, adsorption resin, an AROFO site, or a molecular sieve is mentioned. It is thought that the main thing of the growth inhibition matter is a hydrogen peroxide, as for an adsorbent, what has peroxidation hydrocracking ability or hydrogen-peroxide removal ability is desirable, and activated carbon is specifically mentioned preferably.
[0015] Especially the configuration of the activated carbon used for this invention has desirable powder-like activated carbon from powder and granular or growth of the bacillus which was excellent although

the shape of a sheet etc. was mentioned and all could be used, and the rate of sporangium-izing being shown.

[0016] The adsorption resin as used in the field of this invention is the cross-linking porosity polymer which meant the porosity polymer which adsorbs the detailed matter, for example, was cast in the shape of a particle, and is the synthetic resin which can adsorb the detailed matter in a water solution efficiently according to the pore structure of arriving at even the interior of a particle. Specifically, it is the Mitsubishi Chemical aromatic series system synthetic-resin adsorbent DIAION. HP20, DIAION HP21, SEPABEADS SP825, SEPABEADS SP850, SEPABEADS SP70, SEPABEADS SP700, permutation aromatic series system synthetic-resin adsorbent SEPABEADS SP207, acrylic synthetic-resin adsorbent DIAION HP2MG etc. can be mentioned.

[0017] Although the concentration of the adsorbent in the culture medium used for this invention will not be limited especially if it is range which attains the effectiveness of this invention, its 0.05 - 5 mass % is desirable to a culture medium. If it is more than 0.05 mass %, adsorption of the growth inhibition matter of a bacillus and the removal effectiveness will be demonstrated enough, and if it is 5% or less, since there is also little adsorption of a nutrient required for growth of a bacillus, the growth facilitatory effect of this bacillus excellent in within the limits will be presented. As the addition approach of the adsorbent used for this invention, you may add in the culture medium before sterilization, and may add to the culture medium after sterilization.

[0018] The salt permitted physiologically is also contained in the glutamic acid said by this invention. Specifically, sodium glutamate, potassium glutamate, glutamic-acid ammonium, the glutamic acid hydrochloride, etc. are mentioned. The concentration in these culture media is 0.2 to 4.0 mass % as glutamic acid, and its 0.4 - 1.0 mass % is desirable at the point which presents more excellent growth of a bacillus and the rate of sporangium-izing.

[0019] It is desirable that the nitrogen source needed for the usual microbial cultivation besides glutamic acid is added by the culture medium used for this invention. As a nitrogen source, sources of organic nitrogen, such as the peptone and meat extract which are used for culture of a microorganism, fish meat extractives, a lactalbumin hydrolyzate, or a yeast extract, are usually mentioned. As the other nitrogen source, sources of inorganic nitrogen, such as ammonia, nitric acids, and those salts, are mentioned. It is desirable that it is below 5.0 mass %, and since the concentration in the culture medium of the nitrogen source used for this invention presents the growth facilitatory effect of the more excellent bacillus, its 0.2 - 4.0 mass % is desirable.

[0020] Various kinds of amino acid is contained in the nitrogen source, and glutamic acid will be added in a culture medium as a result by adding a nitrogen source. Therefore, although increasing the addition of this nitrogen source can also raise the concentration of glutamic acid, by the approach, the sporangium which contains a spore and the Pallas PORARU body as a result cannot be formed. In addition to this, this is guessed for the growth inhibition matter contained in a nitrogen source and unnecessary constituent concentration also increasing in coincidence. Therefore, the rate of glutamic acid to all the amino acid in a culture medium has desirable 35 - 90 mass %.

[0021] However, in this invention, all amino acid shall point out the set of 16 kinds of isolation mold amino acid which consists of the alanine by which being contained in the nitrogen source used for usual culture media, such as a peptone and a yeast extract, is known, an arginine, an aspartic acid, glutamic acid, a glycine, an isoleucine, a leucine, a lysine, a methionine, a phenylalanine, a proline, a serine, a threonine, histidines, thyrosins, and valines. The total quantity of the isolation mold amino acid of these 16 classes is often used as what shows in general all the amounts of isolation mold amino acid contained in a peptone, a yeast extract, etc.

[0022] Furthermore, the carbon source needed for the usual microbial cultivation may be added by the culture medium used for this invention. Saccharides, such as trehalose and shoe cloth, are mentioned as a carbon source. Moreover, agricultural production trash, such as blackstrap molasses, a starch decomposition product, and a cheese whey, can also be used. The addition concentration of these carbon sources will not be limited especially if there is in the range which attains the effectiveness of this invention, but since it presents the growth facilitatory effect of the more excellent bacillus, its 0.001 - 5

mass % is desirable to a culture medium. However, in order to make the sporangium containing a spore and the Pallas PORARU body form, as for the glucose concentration contained in a culture medium, it is desirable to carry out to below 0.01 mass % to a culture medium preferably [existence of a glucose].
[0023] The mineral salt of phosphate, such as a potassium dihydrogenphosphate and potassium phosphate, or the sodium salt of those may be added by the culture medium used for this invention if needed. Although the addition concentration of this mineral salt will not be restricted especially if it is range which attains the effectiveness of this invention, below its 1 mass % is desirable to a culture medium.
[0024] The growth of a bacillus and the rate of sporangium-izing which were more excellent in furthermore adding a pyruvic acid to a culture medium can be obtained. The salt of a pyruvic acid permitted physiologically is included in the pyruvic acid said by this invention. As a salt of a pyruvic acid permitted physiologically, pyruvic-acid sodium, a pyruvic-acid potassium, etc. are mentioned concretely.
[0025] The concentration of a pyruvic acid is 0.01 to 0.5 mass % to a culture medium, and is 0.03 to 0.3 mass % to a culture medium at the point which presents more excellent growth of a bacillus and the rate of sporangium-izing preferably. The pyruvic acid added may be sterilized with a culture-medium component, it may divide with a culture-medium component, and it may be sterilized, and may be added at the time of culture initiation.
[0026] The culture medium used for the manufacture approach of this invention may be a liquid medium, or may be a solid medium. In case the manufacture approach of this invention is applied to a liquid medium, water shall also be contained as a culture-medium component. Moreover, as a base material used in case the manufacture approach of this invention is applied to a solid medium, polysaccharide, such as an agar, is mentioned preferably, for example. The concentration in the culture medium of this base material is 0.5 to 5 mass %, and since it presents the growth facilitatory effect of the more excellent bacillus, its 1 - 3 mass % is desirable.
[0027] The temperature suitable for growth of the bacillus belonging to bacillus POPIRIE used for this invention is 25-32 degrees C. Moreover, pH is 6.5-8.5 and is 7-8 more preferably. Alkali usually used, such as acids usually used, such as the buffer solution, and various kinds of hydrochloric acids or sulfuric acids, or a sodium hydroxide, a potassium hydroxide, or ammonia, can be used for adjustment of pH.
[0028] Which approaches, such as batch culture, continuous culture, semibatch culture, or feeding culture, may be used for liquid culture. In the case of batch culture, although culture time amount changes with the culture approach, culture temperature, Culture pH, or inoculation cell mass, it is usually five - ten days.
[0029] What is necessary is to make the buffer solutions, such as water or a phosphate buffer solution, and Tris-HCl, add and suspend, to flush this fungus body, to dissociate and just to collect by general approaches, such as centrifugal separation and filtration, after that, since the fungus body containing this sporangium is on the surface of a culture medium as an approach of collecting the sporangia containing a spore and the Pallas PORARU body from a culture after culture termination in the case of solid culture. What is necessary is in the case of liquid culture, to separate the fungus body containing this sporangium and just to collect from culture medium by the general separation approaches, such as centrifugal separation and filtration. Under the present circumstances, the washing actuation using water or the buffer solution may be added if needed.
[0030] The percentage of the number of sporangia of per the number of bacilli which most sporangia of bacillus POPIRIE which contains in the Scarabaeidae insect the spore which has the prevention effectiveness, and the Pallas PORARU body in culture by the conventional culture medium are not obtained, but is shown at the rate of sporangium-izing of a formula 1 is less than 0.05%.
(Formula 1) rate (%) of sporangium-izing =[(number of sporangia) / (number of bacilli)] x -- 100 [0031] On the other hand, according to the manufacture approach of this invention, it is possible to manufacture the sporangium of bacillus POPIRIE containing a spore and the Pallas PORARU body at 5 - 50% of rate of sporangium-izing. Moreover, it is possible to usually manufacture the number of the sporangia which

contain per 1ml of culture medium, a spore, and the Pallas PORARU body by liquid culture by 5x107 to 1x109 pieces 5x107 or more.
[0032] As a strain which shows growth inhibition or insect-killing activity to the larva of the Scarabaeidae insect also in the strain belonging to bacillus POPIRIE (Bacillus popilliae) bacillus POPIRIE SEMADARA (it popilliaeSemadara(s) Bacillus [ ] --) FERM P-16818 and a bacillus POPIRIE bean (it Mame(s) Bacillus popilliae var. popilliae [ ] --) FERM P-17661 and bacillus POPIRIE HIME (it Hime(s) Bacillus popilliae var. popilliae [ ] --) FERM P-17660 and a bacillus POPIRIE cherry (it Sakura (s) Bacillus popilliae var. popilliae [ ] --) FERM P-17662 and bacillus POPIRIE DEYUTOKI (it Dutk (ies) Bacillus popilliae [ ] --) ATCC No.14706, bacillus POPIRIE MERORONSA (Bacillus popilliae subsp. melolonthae), etc. are mentioned.
[0033] The sporangium of bacillus POPIRIE containing the spore obtained by the manufacture approach of this invention and the Pallas PORARU body shows the prevention effectiveness, such as growth control of insect-killing activity or a larva, to the Scarabaeidae insect. For this reason, this sporangium is useful as a prevention agent of the Scarabaeidae insect.
[0034] As for the Scarabaeidae insect for prevention, a DOUGANE buoy buoy (Anomala cuprea), SEMADARAKOGANE (Blitopertha orientalis), a Japanese beetle (Popillia japonica), USUCHAKOGANE (Phyllopertha diversa), tea IROKOGANE (Adoretustenuimaculatus), a soybean beetle (Anomala rufocuprea), etc. are mentioned.
[0035] You may use as a prevention agent of an insect, especially the Scarabaeidae insect with the liquid which suspended them, or it dries, and the sporangium containing the spore manufactured by the manufacture approach of this invention and the Pallas PORARU body may be used as powder, and may be sprinkled. Moreover, after drying, you may sprinkle as suspension of water or the buffer solution. Furthermore, this sporangium may be pharmaceutical-preparation-ized according to the manufacture approach of the usual microbial pesticide to powder material, a granule, water dispersible powder, an emulsion, liquids and solutions, a floor bull, or the paint with various kinds of additives, such as the support of well-known common use used for agricultural chemicals, a binder, a dispersant, an antifreezing agent, a thickener, or a nutrient. Moreover, it is also possible to mix and use the sporangium containing the spore obtained by the manufacture approach of this invention and the Pallas PORARU body for other microorganism pharmaceutical preparation.
[0036] Although the content rate of the sporangium containing the spore contained in said prevention agent and the Pallas PORARU body changes with the configurations and operation of said prevention agent, its 0.0001 - 100 mass % is usually desirable.
[0037] As the use approach, it is suitably chosen with object crops, such as a pharmaceutical form and operation, etc., for example, approaches, such as ground antiseptic sprinkling, ground solid spraying, air antiseptic sprinkling, air solid spraying, use in a facility, soil incorporation use, or soil douche use, can be mentioned. Moreover, it is also possible to alternation-use or coincidence use it, without mixing with other drugs, i.e., an insecticide, a nematicide, miticide, a herbicide, a germicide, a plant growth regulator, fertilizer, or soil conditioners (peat, humic acid materials, or polyvinyl alcohol system materials), and using or mixing.
[0038] the amount of application of the sporangium which it cannot generally ****** since the amount of application of said prevention agent changes with the class of Scarabaeidae insect, the class of application vegetation, pharmaceutical forms, etc., but contains the spore and the Pallas PORARU body of this invention, for example when carrying out a ground application -- 1010 - 1015 piece /a -- what is necessary is just to make it become about 1011-1014 pieces / a preferably
[0039]
[Example] Hereafter, although an example and the example of a trial explain this invention still more concretely, the range of this invention is not limited to these.
[0040] (Example 1 of reference) The isolation mold amino acid content in the peptone used as a culture-medium component of the culture medium prepared in each example, a yeast extract, and a lactalbumin hydrolyzate was measured with the post column labeling using an alt.phthalaldehyde (OPA).
[0041] (1) The amino acid mixing standard-solution H mold (the Wako Pure Chem make and each

amino acid 2.5 mmol/l are included) was diluted with 0.02M hydrochloric acid 5 times as a preparation standard sample of a sample, it filtered with the pore size 0.2micrometer filter, and the standard sample solution was prepared.
[0042] The test portion prepared the 1.0 mass % solution for the thing of "the poly peptone S" (Japanese-made medicine company make) and "trypton" (Difco make) respectively as a yeast extract, using the thing by the product made from OKUSOIDO, and Difco, and a lactalbumin hydrolyzate (Wako Pure Chem make) as a peptone, and in the 10 mass % trichloroacetic-acid water solution, two fold serial dilution and after agitating well, it removed insoluble precipitate for these according to centrifugal separation. Then, supernatant liquid was filtered with the pore size 0.2micrometer filter, and each test portion solution was prepared.
[0043] (2) 10microl of an analysis standard sample solution and each test portion solution was poured into high performance chromatography, and amino acid analysis was performed. In addition, analysis used the Hitachi amino acid automatic analyzer "LaChrom", and based and went to the passage Fig. shown in drawing 2 . In addition, the presentation of the reaction mixture for OPA indicators and the eluate which were used for this amino analysis was indicated to Tables 1 and 2.
[0044]
[Table 1]

ＯＰＡ標識用反応液の組成	R1	R2	R3
ホウ酸		21.6g	21.6g
水酸化ナトリウム	24.0g		
25%Brij-35溶液		4.0ml	4.0ml
オルトフタルアルデヒド/メチルアルコール			800mg/10ml
2-メルカプトエタノール			2.0ml
5%次亜塩素酸ナトリウム溶液		150.0μl	
全量(蒸留水で1.0Lとした)	1.0L	1.0L	1.0L

[0045] All reagents used the Wako Pure Chem best article.
[0046]
[Table 2]

溶離液の組成	A	B	C
クエン酸ナトリウム $2H_2O$	8.14g	26.67g	
塩化ナトリウム	7.07g	54.35g	
クエン酸 H_2O	20.00g	6.10g	
水酸化ナトリウム			8.0g
エチルアルコール	110ml		
カプリル酸	0.1ml	0.1ml	0.1ml
全量（蒸留水で1.0Lとした）	1.0L	1.0L	1.0L

[0047] All reagents used the Wako Pure Chem make, sodium-citrate 2H2O, citric-acid H2O, and a caprylic acid used the object for amino acid analysis, and others used the best article.
[0048] The content of the L-glutamic acid contained in each test portion and all amino acid was computed by having converted from the peak area obtained from a standard sample solution and each test portion solution, and it was shown in Table 3.
[0049]
[Table 3]

	ペプトン		酵母エキス		ラクトアルブミン
	ポリペプトンS	トリプトン	オクソイド社製	ディフコ社製	水解物
グルタミン酸含有率(質量%)	0.70	1.27	7.74	7.48	2.56
全アミノ酸の合計の含有率(質量%)	17.88	21.65	36.67	31.45	27.37

[0050] Culture-medium conditions given in Hines and others (Journal of Invertebrate pathology, 22 volumes, 377 - 381 pages, 1973), (Example 2 of reference) By namely, the liquid medium containing peptone 0.5 mass %, yeast extract 1.5 mass %, potassium-phosphate 0.3 mass %, glucose 0.1 mass %, and activated carbon 1 mass % The content of the glutamic acid in the culture medium in class setting [which is marketed] each peptone and a yeast extract and the content of the glutamic acid contained in [all] amino acid were calculated, and it was shown in Table 4.

[0051]

[Table 4]

	使用したペプトン 0.5質量%	使用した酵母エキス 1.5質量%	培地中のグルタミン酸の含有率(質量%)	全アミノ酸に対するグルタミン酸の含有率(質量%)
No. 1	ポリペプトンS	オクソイド社製	0.12	18.70
No. 2	ポリペプトンS	ディフコ社製	0.12	20.62
No. 3	トリプトン	オクソイド社製	0.12	18.57
No. 4	トリプトン	ディフコ社製	0.12	20.44

[0052] The content of the glutamic acid in a culture medium was 0.12 mass [at the time of using a peptone (the "trypton" by Difco), and a yeast extract (product made from OKUSOIDO)] %, when a commercial peptone and a commercial yeast extract with the highest glutamic-acid concentration were used.

[0053] Moreover, the content of the glutamic acid similarly contained in [all] amino acid was 20.6 mass [at the time of using a peptone (the "poly peptone S" by the Japanese-made medicine company), and a yeast extract (Difco make)] %, when the commercial high peptone and commercial high yeast extract of the glutamic acid most contained in [all] amino acid were used.

[0054] (An example 1, example 1 of a comparison) the amount which put 80g of distilled water into the preparation flask of a solid medium, and showed L-glutamic acid (Wako Pure Chem best), an adsorbent, a peptone (the "poly peptone S" by the Japanese-made medicine company), a yeast extract (product made from OKUSOIDO), trehalose dihydrate (Wako Pure Chem best), and an agar (Wako Pure Chem best) in Table 5 -- it mixed. Agitating furthermore, the potassium-hydroxide water solution of 1 mol/l was added, and pH was adjusted to 8.0. Furthermore distilled water was added, finally it was referred to as 100g, and - (B-4) was prepared as an example as a culture medium (A-1) and (A-2) an example of a comparison (B-1). in addition, in the activated carbon used as an adsorbent, the Wako Pure Chem best and synthetic adsorption resin used "DIAION HP20" by Mitsubishi Chemical (the following -- the same).

[0055]

[Table 5]

培地名		実施例		比較例			
		A-1	A-2	B-1	B-2	B-3	B-4
培地成分	L-ｸﾞﾙﾀﾐﾝ酸 (g)	0.5	0.5	0.5	—	—	—
	吸着剤 (g)	活性炭 0.1	合成吸着樹脂 2.0	—	活性炭 0.1	合成吸着樹脂 2.0	—
	ﾍﾟﾌﾟﾄﾝ (g)	0.5	0.5	0.5	0.5	0.5	0.5
	酵母ｴｷｽ (g)	0.5	0.5	0.5	0.5	0.5	0.5
	ﾄﾚﾊﾛｰｽ二水和物 (g)	0.1	0.1	0.1	0.1	0.1	0.1
	寒天	2.0	2.0	2.0	2.0	2.0	2.0
全量(g) (蒸留水で100gとした)		100					

[0056] Based on the example 1 of reference, it asked for the rate of glutamic acid to the content and all the amino acid of glutamic acid to a culture medium, respectively, and the result was indicated to Table 6 and 7.

[0057] (An example 2, example 2 of a comparison) Before it sterilized example of culture each culture medium using a solid medium with 121 degrees C and the autoclave for 20 minutes and the agar solidified, it agitated enough, 20ml was poured distributively on each plastics petri dish with a diameter of 9cm, and plate agar was produced.

[0058] The sporangium extracted from the milky disease infection scarab beetle larva was used for the seed fungus of bacillus POPIRIE SEMADARA and a bacillus POPIRIE cherry. The number of sporangia was measured with the direct speculum under a microscope, and sporangium liquid was prepared so that the concentration of a sporangium might be set to ml in 1x107 pieces /with distilled water. These were taken 0.5ml to the plastic tube, and the heat block performed 70 degrees C and heat-treatment for 20 minutes. It applied to the plate agar which prepared 50microl of this seed fungus above, and cultivated for eight days within the 30-degree C culture apparatus.

[0059] 2ml of distilled water was dropped at the petri dish after culture termination, the generated colony was suspended well, and fungus bodies were collected. The number of sporangia and the number of bacilli were measured with the direct speculum under a microscope, and the rate of sporangium-izing was computed using the formula 1. The number of sporangia and the rate of sporangium-izing per petri dish of each strain are shown in Table 6 and 7.

[0060]

[Table 6]

バチルス・ポピリエ・セマダラの培養

培地名	培地に対するｸﾞﾙﾀﾐﾝ酸の含有率（質量%）	全ｱﾐﾉ酸に対するｸﾞﾙﾀﾐﾝ酸の割合（質量%）	胞子嚢数（個/ｼｬｰﾚ）	胞子嚢化率（%）
A-1	0.54	70.16	2.5×10^{9}	42
A-2	0.54	70.16	1.0×10^{9}	37
B-1	0.54	70.16	0	0
B-2	0.04	15.47	5.0×10^{8}	13
B-3	0.04	15.47	2.0×10^{8}	11
B-4	0.04	15.47	0	0

[0061]
[Table 7]
バチルス・ポピリエ・サクラの培養

培地名	培地に対するｸﾞﾙﾀﾐﾝ酸の含有率（質量%）	全ｱﾐﾉ酸に対するｸﾞﾙﾀﾐﾝ酸の割合（質量%）	胞子嚢数（個/ｼｬｰﾚ）	胞子嚢化率（%）
A-1	0.54	70.16	3.5×10^{9}	40
A-2	0.54	70.16	2.0×10^{9}	25
B-1	0.54	70.16	0	0
B-2	0.04	15.47	1.0×10^{9}	18
B-3	0.04	15.47	7.0×10^{8}	9
B-4	0.04	15.47	0	0

[0062] The direction at the time of cultivating each strain from the result of Table 6 and 7 by the culture medium which added glutamic acid under existence of an adsorbent had the number of sporangia, and the high rate of sporangium-izing.
[0063] (An example 3, example 3 of a comparison) the amount which put 700g of distilled water into the preparation flask of a liquid medium, and showed a peptone (the "poly peptone S" by the Japanese-made medicine company), a yeast extract (product made from OKUSOIDO), and trehalose dihydrate (Wako Pure Chem best) for L-glutamic acid (Wako Pure Chem best) or L-alanine (Wako Pure Chem best) in Table 8 further as amino acid to add -- it mixed. The potassium-hydroxide water solution of 5 mol/l was added, pH was adjusted to 7.6, stirring furthermore, distilled water was added further, and, finally it could be 850g. This culture medium was moved to the fermenter (B.E. MARUBISHI Co., Ltd. make) equipped with the pH electrode, and 121 degrees C and autoclave sterilization for 60 minutes were performed.
[0064] Next, activated carbon powder (Wako Pure Chem best) was shown in the flask in Table 8, and distilled water was added further, it was referred to [amount addition was carried out, and] as 100g, and activated carbon dispersion liquid were prepared. Moreover, the defoaming agent ("De Dis home CA-123" by Nippon Oil & Fats Co., Ltd.) was shown in the flask in Table 8, and distilled water was added, it was referred to [amount addition was carried out, and] as 50g, and defoaming agent liquid was prepared. Activated carbon dispersion liquid and defoaming agent liquid were sterilized, to the after-fermentation tub, in sterile, in addition, the culture medium (C-1) was prepared as an example, and culture-medium (D-1) - (D-3) was prepared as an example of a comparison.

[0065]
[Table 8]

培地名		実施例	比較例		
		C-1	D-1	D-2	D-3
培地成分	添加したｱﾐﾉ酸5g	L-ｸﾞﾙﾀﾐﾝ酸	L-ｸﾞﾙﾀﾐﾝ酸	—	L-ｱﾗﾆﾝ
	活性炭(g)	3	—	3	3
	ﾍﾟﾌﾟﾄﾝ(g)	5	5	5	5
	酵母ｴｷｽ(g)	5	5	5	5
	ﾄﾚﾊﾛｰｽ二水和物(g)	5	5	5	5
	消泡剤(g)	1	1	1	1
全量(g) (蒸留水で1000gとした)		1000			

[0066] In order to compare with "Hines and others (Journal of Invertebrate pathology, 22 volumes, 1973, 377 - 381 pages)", (Example 4 of a comparison) 80g of distilled water is put into a flask. Further A peptone (the "trypton" by Difco), the amount which showed a yeast extract (product made from OKUSOIDO), the potassium phosphate (Wako Pure Chem best), a glucose (Wako Pure Chem best), and activated carbon powder (Wako Pure Chem best) in Table 9 -- it mixed. Furthermore, distilled water was added and, finally it could be 100g. This is called a culture medium (D-4). 121 degrees C and autoclave sterilization for 20 minutes were performed.
[0067]
[Table 9]

培地名		比較例
		D-4
培地成分	添加したｱﾐﾉ酸	—
	活性炭(g)	1.0
	ﾄﾘﾌﾟﾄﾝ(g)	0.5
	酵母ｴｷｽ(g)	1.5
	ｸﾞﾙｺｰｽ(g)	0.1
	ﾘﾝ酸水素二ｶﾘｳﾑ(g)	0.3
全量(g)(蒸留水で100gとした)		100

[0068] (An example 4, example 5 of a comparison) The sporangium produced by the culture using the culture medium (A-1) which contains activated carbon beforehand respectively as a seed fungus of example bacillus POPIRIE SEMADARA of culture and the bacillus POPIRIE cherry using a liquid medium, and a bacillus POPIRIE bean was used. The sporangium collected in sterile was measured with the direct speculum under a microscope, and sporangium liquid was prepared so that the concentration of a sporangium might be set to ml in 1x109 pieces /with distilled water.
[0069] 1ml of sporangium liquid of each strain was poured distributively to each plastic tube, and the heat block performed 70 degrees C and heat-treatment for 20 minutes. To a culture medium (C-1) and (D-1) - (D-3), it inoculated each 1ml of sporangium liquid, and it was cultivated for seven days on condition that churning 150rpm, aeration 1vvm, 30 degrees C, and pH7.6 control. On the other hand, the culture medium (D-4) inoculated 0.01ml of sporangium liquid, and within the 30-degree C culture

apparatus, it was agitated at the rotational frequency of 100rpm and it cultivated it for seven days.
[0070] The number of sporangia and the number of bacilli per unit volume after culture termination and in culture medium were measured with the direct speculum under a microscope, and the rate of sporangium-izing was computed using the formula 1. The number of sporangia and the rate of sporangium-izing per 1ml of culture medium are shown in Tables 10-12.
[0071]
[Table 10]
バチルス・ポピリエ・セマダラの培養

培地名	培地に対するグルタミン酸の含有率（質量%）	全アミノ酸に対するグルタミン酸の割合（質量%）	胞子嚢数（個/ml）	胞子嚢化率（%）
C-1	0.54	70.16	1.2×10^8	6.0
D-1	0.54	70.16	0	0
D-2	0.04	15.47	0	0
D-3	0.04	5.76	0	0
D-4	0.12	18.60	0	0

[0072]
[Table 11]
バチルス・ポピリエ・サクラの培養

培地名	培地に対するグルタミン酸の含有率（質量%）	全アミノ酸に対するグルタミン酸の割合（質量%）	胞子嚢数（個/ml）	胞子嚢化率（%）
C-1	0.54	70.16	1.5×10^8	6.8
D-1	0.54	70.16	0	0
D-2	0.04	15.47	0	0
D-3	0.04	5.76	0	0
D-4	0.12	18.60	0	0

[0073]
[Table 12]
バチルス・ポピリエ・マメの培養

培地名	培地に対するグルタミン酸の含有率（質量%）	全アミノ酸に対するグルタミン酸の割合（質量%）	胞子嚢数（個/ml）	胞子嚢化率（%）
C-1	0.54	70.16	1.6×10^8	7.2
D-1	0.54	70.16	0	0
D-2	0.04	15.47	0	0
D-3	0.04	5.76	0	0
D-4	0.12	18.60	0	0

[0074] The sporangium was obtained only in the culture medium which added an adsorbent and glutamic acid so that clearly from the result of Tables 10-12.

[0075] (An example 5, example 6 of a comparison) the amount which put 700g of distilled water into the example beaker of preparation of a liquid medium, and showed L-glutamic acid (Wako Pure Chem best), a peptone (the "poly peptone S" by the Japanese-made medicine company), a yeast extract (product made from OKUSOIDO), a lactalbumin hydrolyzate (Wako Pure Chem make), and trehalose dihydrate (Wako Pure Chem best) in Table 13 -- it mixed. The potassium-hydroxide water solution of 5 mol/l was added, pH was adjusted to 7.6, stirring, distilled water was added further, and it could be 850g. This culture medium was moved to the fermenter (B.E. MARUBISHI Co., Ltd. make) equipped with the pH electrode, and 121 degrees C and autoclave sterilization for 60 minutes were performed.
[0076] Next, activated carbon powder (Wako Pure Chem best) was shown in the flask in Table 13, and distilled water was added, it was referred to [amount addition was carried out, and] as 100g, and activated carbon dispersion liquid were prepared. Moreover, the defoaming agent ("De Dis home CA-123" by Nippon Oil & Fats Co., Ltd.) was shown in the flask in Table 13, and distilled water was added further, it was referred to [amount addition was carried out, and] as 50g, and defoaming agent liquid was prepared. these activated carbon dispersion liquid and defoaming agent liquid are sterilized, and sterile [to each fermenter] after that ---like -- adding -- further -- distilled water -- adding -- final -- 1000g -- carrying out -- as an example -- as the example of a culture medium (E-2) - (E-6) a comparison -- a culture medium (E-1) -- and (E-7) it prepared.
[0077]
[Table 13]

培地名		比較例	実施例					比較例
		E-1	E-2	E-3	E-4	E-5	E-6	E-7
培地成分	L-ｸﾞﾙﾀﾐﾝ酸 (g)	-	0.2	0.5	0.8	1.5	3.0	5.0
	活性炭 (g)	3	3	3	3	3	3	3
	ﾍﾟﾌﾟﾄﾝ (g)	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	酵母ｴｷｽ (g)	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	ﾗｸﾄｱﾙﾌﾞﾐﾝ水解物(g)	5	5	5	5	5	5	5
	ﾄﾚﾊﾛｰｽ二水和物 (g)	5	5	5	5	5	5	5
	消泡剤(g)	1	1	1	1	1	1	1
全量(g) (蒸留水で1000gとした)		1000						

[0078] (An example 6, example 7 of a comparison) The sporangium produced by the culture using the culture medium (A-1) which contains activated carbon beforehand respectively as a seed fungus of example bacillus POPIRIE SEMADARA of culture using a liquid medium was used. The sporangium collected in sterile was measured with the direct speculum under a microscope, and sporangium liquid was prepared so that the concentration of a sporangium might be set to ml in 1x109 pieces /with distilled water.
[0079] 1ml of sporangium liquid was poured distributively to each plastic tube, and the heat block performed 70 degrees C and heat-treatment for 20 minutes. This was inoculated into each culture medium 1ml, and was cultivated for seven days on condition that churning 150rpm, aeration 1vvm, 30 degrees C, and pH7.6 control. The number of sporangia and the number of bacilli per unit volume after culture termination and in culture medium were measured with the direct speculum under a microscope, and the rate of sporangium-izing was computed using the formula 1. The cell number, the number of sporangia, and the rate of sporangium-izing per 1ml of culture medium are shown in Table 14. Moreover, the relation of the glutamic-acid concentration (mass %), the number of bacilli (x108 piece/ml), and the number of sporangia (x107 piece/ml) to a culture medium is shown in drawing 3 .

[0080]
[Table 14]

培地名	培地に対するｸﾞﾙﾀﾐﾝ酸の含有率 (質量%)	全ｱﾐﾉ酸に対するｸﾞﾙﾀﾐﾝ酸の割合 (質量%)	菌体数 (個/ml)	胞子嚢数 (個/ml)	胞子嚢化率 (%)
E-1	0.08	13.94	5.8×10^{8}	0	0
E-2	0.28	37.01	6.9×10^{8}	9.6×10^{7}	14.0
E-3	0.58	55.08	1.1×10^{9}	1.8×10^{8}	17.0
E-4	0.88	65.09	1.3×10^{9}	1.7×10^{8}	12.7
E-5	1.58	77.03	6.8×10^{8}	9.2×10^{7}	13.5
E-6	3.08	86.75	4.6×10^{8}	5.2×10^{7}	11.2
E-7	5.08	91.53	3.5×10^{8}	0	0

[0081] (An example 7, example 8 of a comparison) the amount which puts 700g of distilled water into the example beaker of preparation of a liquid medium, and shows L-glutamic acid (Wako Pure Chem best), pyruvic-acid sodium (Wako Pure Chem best), a peptone (the "poly peptone S" by the Japanese-made medicine company), a yeast extract (product made from OKUSOIDO), a lactalbumin hydrolyzate (Wako Pure Chem make), and trehalose dihydrate (Wako Pure Chem best) in Table 15 -- it mixed. Then, the four mols [/l.] sodium-hydroxide water solution was added, pH was prepared to 7.6, agitating, distilled water was added further, and, finally it could be 850g. The prepared culture medium was put into the fermenter (B.E. MARUBISHI Co., Ltd. make) equipped with the pH electrode, and 121 degrees C and autoclave sterilization for 50 minutes were performed.
[0082] Next, activated carbon powder (Wako Pure Chem best) was shown in the flask in Table 15, and distilled water was added further, it was referred to [amount addition was carried out, and] as 100g, and activated carbon dispersion liquid were prepared. Moreover, the defoaming agent ("De Dis home CA-123" by Nippon Oil & Fats Co., Ltd.) was shown in the flask in Table 15, and distilled water was added further, it was referred to [amount addition was carried out, and] as 50g, and defoaming agent liquid was prepared. These activated carbon dispersion liquid and defoaming agent liquid were sterilized, and, in addition, the culture medium (F-3) was prepared [to the after-fermentation tub] as an example as a culture medium (F-1) and (F-2) an example of a comparison in sterile.
[0083]
[Table 15]

培地名		実施例		比較例
		F-1	F-2	F-3
培地成分	L-ｸﾞﾙﾀﾐﾝ酸 (g)	5.0	5.0	-
	ﾋﾟﾙﾋﾟﾝ酸ﾅﾄﾘｳﾑ (g)	1.0	2.5	1.0
	活性炭 (g)	2.5	2.5	2.5
	ﾍﾟﾌﾟﾄﾝ (g)	7.5	7.5	7.5
	酵母ｴｷｽ (g)	7.5	7.5	7.5
	ﾗｸﾄｱﾙﾌﾞﾐﾝ水解物 (g)	5	5	5
	ﾄﾚﾊﾛｰｽ二水和物 (g)	5	5	5
	消泡剤	1	1	1
全量(g) (蒸留水で1000gとした)		1000		

[0084] (An example 8, example 9 of a comparison) Like the example example 6 of culture using a liquid medium, using bacillus POPIRIE SEMADARA as a seed fungus, inoculation of every 1ml each was carried out to culture-medium (F-1) - (F-3) in sterile, and culture was started. The culture condition set to the temperature of 29 degrees C, quantity-of-airflow 0.5vvm, and rotational frequency 150rpm, and was controlled by the sodium-hydroxide solution of 4 mol/l, and the sulfuric-acid water solution of 4 mol/l to pH7.6 during culture.

[0085] It cultivated for five days, the number of sporangia and the number of bacilli per unit volume in culture medium were measured with the direct speculum under a microscope, and the rate of sporangium-izing was computed. The number of a culture medium (F-1) - (F-3) bacilli, the number of sporangia, and the rate of sporangium-izing were shown in Table 16.

[0086]

[Table 16]

培地名	培地に対するｸﾞﾙﾀﾐﾝ酸の含有率 (質量%)	全ｱﾐﾉ酸に対するｸﾞﾙﾀﾐﾝ酸の割合(質量%)	ﾋﾟﾙﾋﾟﾝ酸含有率(質量%)	菌数 (個/ml)	胞子嚢数 (個/ml)	胞子嚢化率(%)
F-1	0.58	55.08	0.08	1.5×10^{9}	2.5×10^{8}	16.7
F-2	0.58	55.08	0.20	1.6×10^{9}	4.8×10^{8}	30.0
F-3	0.06	15.47	0.08	1.0×10^{9}	0	0

[0087] It became a high rate of sporangium-izing by adding pyruvic-acid sodium and controlling pH, and the obtained number of sporangia was also high.

[0088] (Example 1 of a bioassay) The growth depressor effect trial of the larva of the Scarabaeidae insect by the sporangium obtained by the manufacture approach of this invention was performed. The sporangium of bacillus POPIRIE SEMADARA acquired by the culture medium using the culture medium (A-1) of an example 2 was made to suspend so that it may become distilled water in 2x108 pieces/[ml and], and suspension (I) was prepared. Furthermore, French press processing of the suspension containing the sporangium of bacillus POPIRIE SEMADARA acquired by the culture medium using the culture medium (A-1) of an example 2 was carried out, and a spore and the Pallas PORARU body were separated and taken out from the sporangium. The separated spore was made to suspend so that it may become distilled water in 2x108 pieces/[ml and], and suspension (II) was prepared. Moreover, the separated Pallas PORARU body was made to suspend so that it may become distilled water in 2x108 pieces/[ml and], and suspension (III) was prepared.

[0089] 80 plastics cups with a diameter of 6cm into which it put about 20g of leaf mold at a time were prepared.
i) The suspension (I) which contains a sporangium so that the number of sporangia may serve as 2x108 pieces / cup was sprinkled to 20 plastics cups.
ii) The suspension (II) which contains only a spore so that the number of spores may serve as 2x108 pieces / cup was sprinkled to 20 plastics cups.
iii) The suspension (III) which contains only the Pallas PORARU body so that the number of the Pallas PORARU bodies may serve as 2x108 pieces / cup was sprinkled to 20 plastics cups.
iv) Nothing was sprinkled to the 20 remaining pieces, but it considered as the control test.
It put one DOUGANE buoy buoy second instar larva at a time into each cup, and bred for 30 days within the 25-degree C culture apparatus, and the augend of the death rate of a larva and the average weight of a survival larva was measured with time. The accumulation death rate is shown in Table 17, and a result is shown in drawing 4 about growth depressor effect.
[0090]
[Table 17]

	累積死亡率 (%)		
試験区	１１日目	２３日目	３０日目
i)	２０	４０	４５
ii)	０	５	１０
iii)	１５	２０	２５
対 照	０	０	０

[0091] Having the insect-killing effectiveness excellent in the sporangium containing a spore and the Pallas PORARU body and the growth depressor effect of a larva was checked from the above result.
[0092] (Example 2 of a bioassay) The insecticidal test of the Scarabaeidae insect by the sporangium obtained by the manufacture approach (solid culture) of this invention was performed.
[0093] The sporangium of bacillus POPIRIE SEMADARA acquired by the culture using the activated carbon content plate agar (A-1) of an example 2 was suspended so that it might become distilled water in 1x109 pieces/[ml and], and sporangium liquid was prepared. It put about 20g of leaf mold at a time into 40 plastics cups with a diameter of 6cm, and to 20 of pieces [them], sporangium liquid was sprinkled so that the number of sporangia might serve as 1x109 pieces / cup. Sporangium liquid was not sprinkled to the 20 remaining pieces, but it considered as the control test. It put one DOUGANE buoy buoy second instar larva at a time into each cup, and bred for 40 days within the 25-degree C culture apparatus, the death population was investigated with time, and the accumulation death rate (%) was searched for.
[0094] The insect-killing activity over the DOUGANE buoy buoy of the sporangium obtained by the solid culture of this invention in Table 18 is shown. 100% of death rate was observed in the 40th day.
[0095]
[Table 18]

	累積死亡率 (%)			
試験区	１０日目	２０日目	３０日目	４０日目
対 照	０	０	０	０
胞子嚢添加	４０	６０	９０	１００

[0096] (Example 3 of a bioassay) The insecticidal test of the Scarabaeidae insect by the sporangium

obtained by the manufacture approach (liquid culture) of this invention was performed. The experimental plot was produced like the example 2 of a bioassay. however, the sporangium of bacillus POPIRIE SEMADARA which acquired the sprinkled sporangium by the culture which used the activated carbon content liquid medium (C-1) of the i example 4 and the sporangium of the bacillus POPIRIE bean acquired by the culture using the activated carbon content liquid medium (C-1) of the ii example 4 -- it came out. It put one DOUGANE buoy buoy second instar larva at a time into each cup, and bred for 40 days within the 25-degree C culture apparatus, the death population was investigated with time, and the accumulation death rate (%) was searched for.

[0097] The insect-killing activity over the DOUGANE buoy buoy of the sporangium obtained by the liquid culture of this invention in Table 19 is shown. 85 - 100% of death rate was observed in the 40th day.

[0098]

[Table 19]

	累積死亡率 (%)			
試験区	１０日目	２０日目	３０日目	４０日目
対 照	0	0	0	0
i)	１５	３０	９５	１００
ii)	１０	３５	６５	８５

[0099] (Example 4 of a bioassay) The insecticidal test of the Scarabaeidae insect by the sporangium obtained by the manufacture approach (liquid culture) of this invention was performed. The sporangium of the bacillus POPIRIE SEMADARA stock obtained by culture of the culture medium (F-2) shown in the example 8 was suspended so that it might become distilled water in 1x109 pieces/[ml and], and sporangium liquid was prepared.

[0100] It put 20g of leaf mold at a time into 40 plastics cups with a diameter of 6cm. To 20 of pieces [them], sporangium liquid was sprinkled so that the number of sporangia might serve as 1x109 pieces / cup. Sporangium liquid was not sprinkled to the 20 remaining pieces, but it considered as the control test. It put one DOUGANE buoy buoy second instar larva at a time into each cup, and bred for 40 days within the 25-degree C culture apparatus, the death population was investigated with time, and the accumulation death rate (%) was investigated.

[0101] The result of the insect-killing activity of the insect outside-of-the-body formation sporangium to a DOUGANE buoy buoy is shown in Table 20. The obtained sporangium showed insect-killing activity and all the larvae died by the 40th.

[0102]

[Table 20]

	累積死亡率 (%)			
試験区	１０日目	２０日目	３０日目	４０日目
対照	0	0	0	0
胞子嚢添加	１５	３０	９５	１００

[0103]

[Effect of the Invention] This invention can offer the manufacture approach of obtaining efficiently the sporangium of bacillus POPIRIE containing a spore and the Pallas PORARU body. That is, this invention is a liquid medium for about five - ten days, and can manufacture the sporangium which can manufacture the sporangium of bacillus POPIRIE containing a spore and the Pallas PORARU body at 5 - 50% of rate of sporangium-izing, and contains per 1ml of culture medium, a spore, and the Pallas

PORARU body at a rate of 5x107 or more pieces. Moreover, this invention can offer the prevention approach of the insect using the prevention agent and this prevention agent which show the prevention effectiveness, such as growth inhibition of insect killing or a larva, to an insect, especially the Scarabaeidae insect, especially the Scarabaeidae insect.

[Translation done.]

CLAIMS

[Claim(s)]
[Claim 1] The manufacture approach of the sporangium containing the spore and the Pallas PORARU body of bacillus POPIRIE which has the prevention effectiveness to the Scarabaeidae insect which is an approach of manufacturing the sporangium which cultivates the bacillus belonging to bacillus POPIRIE by the culture medium, and contains a spore and the Pallas PORARU body, and is characterized by cultivating glutamic acid by the culture medium 0.05-0.5 mass % Containing 0.2 to 4.0 mass %, and an adsorbent.
[Claim 2] The manufacture approach of a sporangium according to claim 1 35-90 mass % That said culture medium contains glutamic acid to all the amino acid in a culture medium.
[Claim 3] The manufacture approach of a sporangium according to claim 1 that said culture medium contains a pyruvic acid in a culture medium.
[Claim 4] The prevention agent of the Scarabaeidae insect which contains the sporangium containing the spore and the Pallas PORARU body of bacillus POPIRIE obtained by the manufacture approach according to claim 1 as an active principle.
[Claim 5] The prevention approach of the Scarabaeidae insect which sprinkles a prevention agent according to claim 4 in the habitation soil of the Scarabaeidae insect.

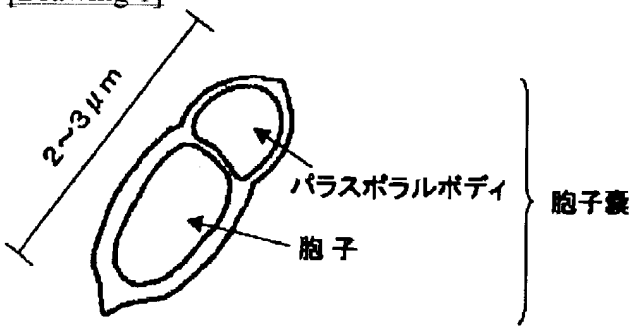
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the mimetic diagram of the sporangium of bacillus POPIRIE containing a spore and the Pallas PORARU body.
[Drawing 2] It is the mimetic diagram of the high-speed liquid chroma TOGURAFISHI stem used for amino acid analysis.
[Drawing 3] It is the graph which showed the number of sporangia and cell number to glutamic-acid concentration in the culture medium in an example 3.
[Drawing 4] It is the graph which showed the growth inhibition effectiveness of the DOUGANE buoy buoy in the example 1 of a bioassay.
[Description of Notations]
1 Sporangium
2 Pallas PORARU Body
3 Spore

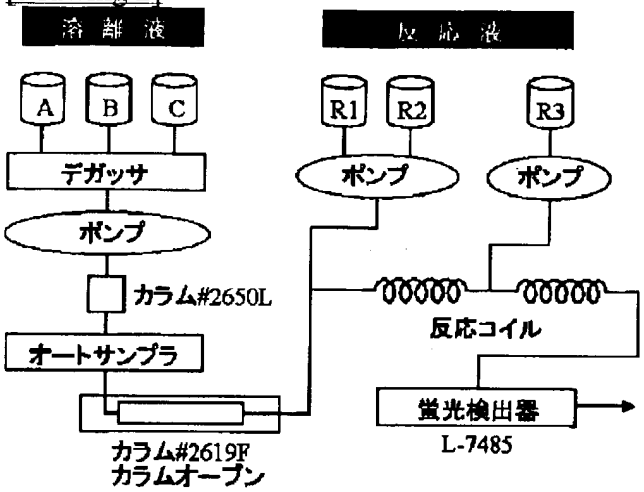
[Translation done.]

DRAWINGS

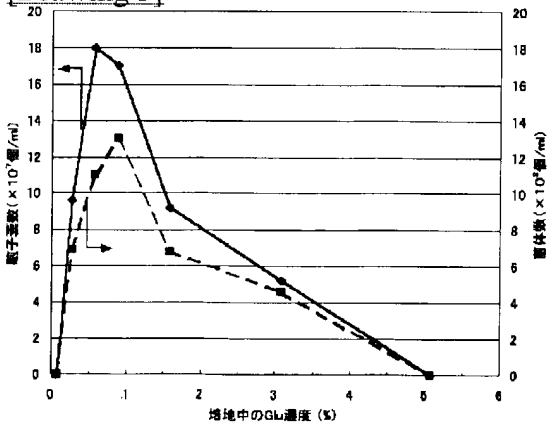
[Drawing 1]



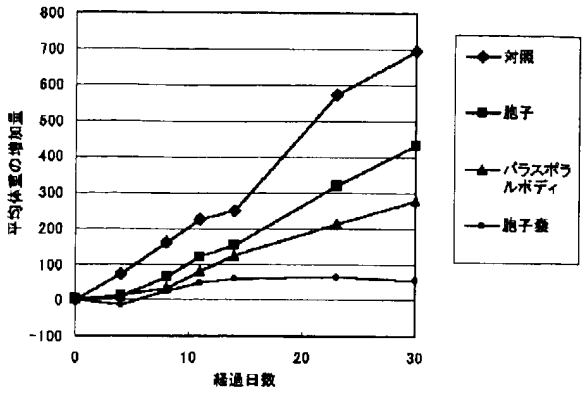
[Drawing 2]



[Drawing 3]



[Drawing 4]



[Translation done.]

CORRECTION OR AMENDMENT

[Kind of official gazette] Printing of amendment by the convention of 2 of Article 17 of Patent Law
[Section partition] The 1st partition of the 1st section
[Publication date] March 11, Heisei 15 (2003. 3.11)

[Publication No.] JP,2002-355030,A (P2002-355030A)
[Date of Publication] December 10, Heisei 14 (2002. 12.10)
[Annual volume number] Open patent official report 14-3551
[Application number] Application for patent 2002-94765 (P2002-94765)
[The 7th edition of International Patent Classification]

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C12N   1/20
A01N  63/02
//(C12N   1/20
C12R   1:07   )
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[FI]

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C12N   1/20        A
A01N  63/02        E
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[Procedure revision]
[Filing Date] August 26, Heisei 14 (2002. 8.26)
[Procedure amendment 1]
[Document to be Amended] Trust changed-number report
[Item(s) to be Amended] Those who took the necessary procedure
[Method of Amendment] Addition
[Proposed Amendment]
[Others] It is indifferent from having carried out this procedure.